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Induction of Mutations by Chemical Agents at the Hypoxanthine-Guanine Phosphoribosyl Transferase Locus in Human Epithelial Teratoma Cells

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16. ABSTRACT

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Induction of mutations by chemical agents at the hypoxanthine–guanine phosphoribosyl transferase locus in human epithelial teratoma cells

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Summary

Induction of 6-thioguanine (TG) resistance by chemical mutagens was examined in a line of cells derived from a human epithelial teratocarcinoma cell clone. The cells, designated as P3 cells, have a stable diploid karyotype with 46(XX) chromosomes, including a translocation between chromosomes 15 and 20. Efficient recovery of TG-resistant mutants induced by the direct-acting mutagens: *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG); 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE); and benzo[*a*]pyrene (B[*a*]P); activated in a cell-mediated assay, required an expression time of 7 days and a saturation density of 2×10^4 cells/60-mm petri dish. The TG-resistant mutant cells induced by MNNG and BPDE maintained their resistant phenotype 4–6 weeks after isolation. This mutant phenotype was associated with a more than 10-fold reduction in hypoxanthine–guanine phosphoribosyl transferase (HGPRT) activity relative to that of the parental P3 cell line, which was shown to catalyze the formation of 4.6 pmoles inosine-5'-monophosphate (IMP)/min/μg protein. Induction of TG resistance was also observed in P3 cells cocultivated in a cell-mediated assay with human breast carcinoma cells, which are capable of polycyclic aromatic hydrocarbon (PAH) metabolism, after treatment with the carcinogenic PAHs: B[*a*]P, chrysene, 7,12-dimethylbenz[*a*]anthracene (DMBA), and 3-methylcholanthrene (MCA). The degree of mutant induction in this assay was related to the carcinogenic potency of these PAHs in experimental animals. The most potent mutagen was DMBA, followed in decreasing order by MCA, B[*a*]P, and chrysene. DMBA, at 0.4 μM, increased the frequency of mutants for TG resistance from 2 for the control to about 200 TG-resistant mutants/10^6 colony-forming cells (CFC). Benzo[*e*]pyrene (B[*e*]P) and pyrene, which are not carcinogenic, were not effective in the assay. None of the PAHs was mutagenic in the P3 cells cultivated in the absence of the PAH-metabolizing cells. These results indicate that the P3 cells can be useful for the study of mutagenesis at the HGPRT locus by direct-acting chemical mutagens, as well as by chemicals activated in a cell-mediated assay.

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A number of investigators have employed human fibroblast strains derived from healthy individuals or from xeroderma pigmentosum patients to study the mutagenic response of UV irradiation and some chemically reactive mutagens. Using such genetic markers as resistance to 8-azaguanine, 6-thioguanine (TG) or diphtheria toxin, these investigators have been able to achieve quantitative responses in the induction of mutations (Glover et al., 1979; Gupta and Siminovitch, 1978; Jacobs and DeMars, 1978; McCormick and Maher, 1981). Human fibroblasts were also used in mutagenesis studies with chemicals that require metabolism in order to express their mutagenic activity (Aust et al., 1980; Michalopoulos et al., 1981; Strom et al., 1981, Tong et al., 1981). However, because of the limited life span of human cells in culture (Hayflick and Moorhead, 1961) and low saturation density, such human fibroblast strains are not frequently used in screening assays for hazardous environmental chemicals. Thilly and his associates (1980) have overcome some of the above limitations by introducing the use of human lymphoid cells, which grow continuously in culture. Like cells from many other cell lines, these lymphoid cells lack the enzymes required for the metabolism of many environmental chemicals, and therefore must be supplemented with a rat-liver subcellular fraction for the metabolic activation of the test chemical. However, subcellular liver fractions will not necessarily metabolize chemicals in a manner analogous to that of intact cells (Bigger et al., 1980; Newbold et al., 1977; Selkirk, 1977). Mutagenesis assays, such as the cell-mediated assay, that use viable normal cells for metabolic activation yield a chemical activation resembling that observed in vivo (Newbold et al., 1977; Sebti et al., 1982). Indeed, studies with polycyclic aromatic hydrocarbons (PAHs), nitrosamines, and aflatoxins have shown that results obtained in cell-mediated mutagenesis assays can reflect cell/organ specificity and the carcinogenic potency of the tested chemicals (Gould, 1980, 1982; Huberman and Sachs, 1976; Huberman and Slaga, 1979; Jones et al., 1981, 1983; Kuroki et al., 1982; Langenbach et al., 1978a, b, 1981a, b; Newbold et al., 1979; Raveh et al., 1982; Reiner et al., 1983). Cells growing attached to the surface of tissue-culture vessels are extremely useful in the cell-mediated mutagenesis assay, which requires contact between the target and metabolizing (mediating) cells for efficient transfer of the active intermediates to the target cells (Kuroki and Drevon, 1978).

In the present study we characterized the usefulness of such cells from a human epithelial teratocarcinoma cell line (designated P3). These P3 cells were used as target cells in mutagenesis studies with direct-acting mutagens/carcinogens, as well as with proximate mutagens/carcinogens activated in a cell-mediated assay using human breast carcinoma cells for chemical metabolism.

Materials and methods

Chemicals

Benzo[*a*]pyrene (B[*a*]P), benzo[*e*]pyrene (B[*e*]P), (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) — which is the ultimate mutagenic form of B[*a*]P (Huberman et al., 1976) — chrysene, 7,12-dimethylbenz[*a*]anthracene (DMBA), 3-methylcholanthrene (MCA), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), pyrene, and 6-thioguanine (TG) were supplied by Sigma Chemical Co., St. Louis, MO, or were obtained from the Research Resources Section of the National Cancer Institute (Bethesda, MD). Prior to their dilution in the growth media, TG and the PAHs were dissolved in dimethyl sulfoxide. BPDE and MNNG, dissolved in anhydrous acetone, were added directly to the cultures. The final concentration of acetone or dimethyl sulfoxide in the growth medium was 0.5%.

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Abbreviations: B[*a*]P, benzo[*a*]pyrene; B[*e*]P, benzo[*e*]pyrene; BPDE, 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene; CFC, colony-forming cells; DMBA, 7,12-dimethylbenz[*a*]anthracene; HGPRT, hypoxanthine-guanine phosphoribosyl transferase; IMP, inosine-5'-monophosphate; MCA, 3-methylcholanthrene; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; PAH, polycyclic aromatic hydrocarbon; TG, 6-thioguanine; trypsin–EDTA, trypsin–ethylenediaminetetraacetate.

Cell culture

The P3 cells, which exhibit an epithelial cell morphology, were derived from a clone isolated from a human teratocarcinoma cell line (Zeuthen et al., 1980) kindly supplied by Dr. P. Giovanella (The Stehlin Foundation for Cancer Research, Houston, TX). The epithelial BJ (BJ-015) cells, also with an epithelial cell morphology, obtained from a pleural effusion of a human breast carcinoma were received from the Cell Culture Laboratory, University of California, Naval Bioscience Laboratory (Naval Supply Center, Oakland, CA). Cells were cultured in RPMI-1640 medium supplemented with 20% fetal calf serum plus penicillin (100 units/ml) and streptomycin (100 μg/ml) (Gibco, Grand Island, NY). Cell numbers were determined after the exclusion of nonviable cells, which were stained by trypan blue.

Determination of hypoxanthine–guanine phosphoribosyl transferase (HGPRT) (E.C.2.4.2.8) activity

Cell-free lysates from the parental P3 cells and the TG-resistant cells were prepared according to the procedure described by Capechi et al. (1974). Cells were seeded at 2×10^6 cells/100-mm petri dish and extracted 3–4 days later. The cultures were rinsed 3 times with 5 ml of ice-cold phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4 (pH 7.5)). Ice-cold lysis buffer (10 mM Tris · HCl) (pH 7.4), 10 mM $MgCl_2$, 30 mM KCl, 0.5% Triton X-100, and 0.1 mM dithiothreitol) in the amount of 0.3 ml was added to each of the petri dishes which were gently shaken every 5 min for 20 min, then drained for 10 min, prior to collection of the lysate. Both the cell lysis and lysate collections were performed at 4°C. An aliquot of the cell lysate was taken for protein determination (Lowry et al., 1951).

HGPRT activity was assayed according to Gillin et al. (1972). Cell-free extracts (6–12 μg protein) were incubated for 10 min at 37°C with 1 mM phosphoribosyl pyrophosphate, 0.1 mM hypoxanthine, 2 μCi [^{3}H]hypoxanthine (690 pmoles), 50 mM Tris · HCl (pH 7.5), 5 mM $MgCl_2$, 10 mM KCl, and 6 mM adenosyl-5′-monophosphate in a final volume of 0.1 ml. The addition of excess adenosyl-5′-monophosphate was found to sufficiently diminish the hydrolysis of the enzymatic product inosine-5′-monophosphate (IMP). The reaction was terminated by the addition of 0.1 ml 95% ethanol. Two assay samples were treated with ethanol prior to the addition of the cell extracts (containing the enzyme activity) to establish the background levels of [^{3}H]IMP. The reaction was linear from 5 to 45 min and from 0.5 to 50 μg protein, after which the activity of 5′-nucleotidase exceeded by 20% that of HGPRT.

The reaction products were separated by descending paper chromatography. Samples (50 μl) and carrier IMP, inosine, and hypoxanthine were spotted on 3-mm paper prior to development in ammonium acetate saturated with sodium borate (pH 9.0) : 95% ethanol, 3 : 7 (v/v). The *rf* values for IMP, inosine, and hypoxanthine were 0.08, 0.44, and 0.67, respectively. Samples were identified under 254-nm ultraviolet light, cut out, and analyzed for radioactivity in a model 6892 Searle liquid scintillation spectrometer.

Chromosome analysis

Exponentially growing parental and mutant P3 cells were harvested after treatment for 1 h with a final concentration of 0.1 μg/ml colcemid (Grand Island Biological Company, Grand Island, NY). The harvested cells were suspended in a hypotonic solution (0.075 M KCl) at 37°C and 10 min later fixed by adding a solution of glacial acetic acid : methanol, 1 : 3 (v/v), which was changed a number of times. The fixed cells were spread on glass slides, air dried, and stained for G-banding as described (Seabright, 1971). For each cell type a total of 50 well-spread metaphase cells were photographed and analyzed for chromosome number and chromosome abnormalities according to the Paris Conference (1972) and International System for Human Cytogenetic Nomenclature (1978).

Induction of 6-thioguanine resistance by chemically reactive mutagens / carcinogens and by chemicals activated in a cell-mediated assay

To test for TG resistance, we treated exponentially growing P3 cells with different concentrations of the mutagens. For each concentration, we added the mutagen in solution to 2–8 100-mm petri dishes, which had been seeded 2 days earlier

with 10^6 cells in 10 ml growth medium. After 3 h of treatment, the cultures were dissociated with a solution of trypsin (0.5 g/l; 1:250)–ethylenediaminetetraacetate (0.2 g/l) (trypsin–EDTA) and the dissociated cells were seeded at 200 cells in 5 ml of growth medium/60-mm petri dish to determine the cloning efficiencies of control and treated P3 cells. These efficiencies are indicative of the toxic effect of the tested chemicals. Cells were also seeded into 10–20 petri dishes at 3×10^5 cells in 10 ml of growth medium/100-mm petri dish to allow an optimal phenotypic expression of the induced mutations (Bradley et al., 1981; Diamond et al., 1982; O'Neill et al., 1977; Van Zeeland and Simons, 1976). At different times after this cell seeding, the cultures were dissociated with trypsin–EDTA and the cells were seeded at 200 cells in 5 ml growth medium/60-mm petri dish to determine cloning efficiencies and at 2×10^4 cells in 5 ml of selective medium containing an optimal concentration of 40 μM TG/60-mm petri dish to determine the number of TG-resistant mutants. The cells used for the zero expression time were from those cultures dissociated immediately after treatment with the mutagen. The cloning efficiencies were determined by counting the number of Giemsa-stained colonies in 6–8 dishes/type of treatment at 10 days after cell seeding, and the number of TG-resistant mutants was determined by counting Giemsa-stained colonies in 36–60 petri dishes/type of treatment at 14–16 days after cell seeding. Mutation frequencies for TG resistance were calculated per 10^6 colony-forming cells (CFC), based on the cloning efficiency and the number of cells seeded for mutant selection.

To test for induction of TG resistance by PAH in the cell-mediated assay, we seeded 10^6 BJ cells (X-ray irradiated with 5000 R) in 3 ml of growth medium into 60-mm petri dishes. The following day, the irradiated cells were overlaid with 1 ml of growth medium containing 3×10^5 P3 cells per petri dish. The PAHs in 1 ml of growth medium were added 5 h later. The cultures were incubated for 2 days, after which the medium was removed. The cultures were then dissociated with trypsin–EDTA and the cells seeded at 200 cells in 5 ml growth medium/60-mm petri dish to determine the cytotoxicity of the tested PAHs. The number of Giemsa-stained colonies was determined 10 days later. The TG-resistant mutants for the various expression times were selected as described for the direct-acting mutagens/carcinogens.

Results

Conditions for the efficient selection of mutagen-induced TG-resistant mutants in P3 cells

To obtain cells with a high cloning efficiency and a short generation time, we isolated a clone of cells designated P3 from a human teratoma cell line, which grows attached to the surface of petri dishes (Zeuthen et al., 1980). The P3 cells exhibit an epithelial cell morphology and are characterized by a cloning efficiency of 65 ± 10% and a generation time of about 16 h, which lead to the production of large countable colonies within 10 days. The P3 cells can be mutated for TG resistance by a number of chemical agents.

In order to establish an optimal cell density for the detection of TG-resistant mutants in control and in mutagen-treated P3 cells, we incubated the cells at different cell densities prior to the addition of TG (Fig. 1). Control cells seeded at 10^5

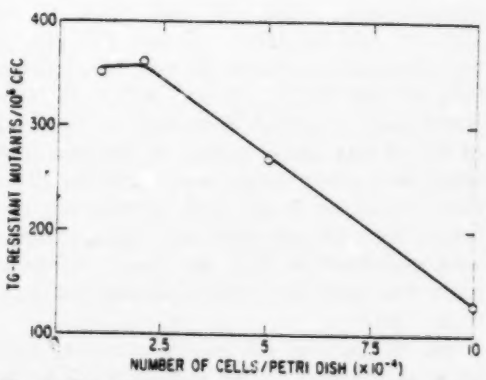

Fig. 1. The recovery of TG-resistant mutants from P3 cells seeded at different cell densities. 7 days after treatment with 7 μM MNNG, the cells were seeded at different cell densities and incubated with TG. The cloning efficiency of the control cells was 68% and of the MNNG-treated cells 61%. Control cells yielded at a seeding level of 10^5 cells/60-mm petri dish < 1 mutant/10^6 CFC. At seeding levels of 5×10^4 and 10^4 cells/dish, 2 mutants/10^6 CFC were found; at a cell density of 2×10^4 cells/dish, 1 mutant/10^6 CFC was found.

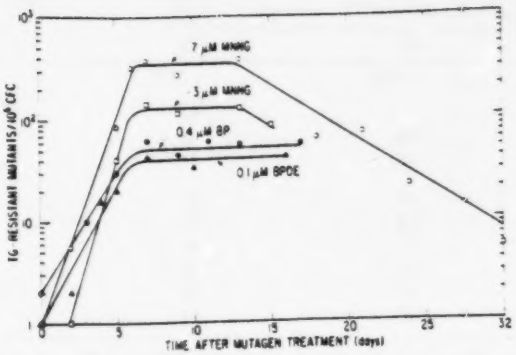

Fig. 2. The recovery of TG-resistant mutants from P3 cells at different times after treatment with MNNG and BPDE and with B[*a*]P activated in a cell-mediated assay with the human breast carcinoma BJ cells. At different times after treatment, the control and mutagen-treated cells were seeded at 2×10^4 cells/60-mm petri dish and incubated with TG. The cloning efficiencies of the control cells varied from 35 to 72% and of the treated cells from 9 to 53%. The lower cloning efficiencies were detected in the early time points (0–4 days). The mutation frequencies of control cells varied from 1 to 2 TG-resistant mutants/10^6 CFC.

cells/petri dish yielded < 1 mutant/10^6 CFC, whereas at the lower cell densities of $1–5 \times 10^4$ cells/petri dish, the frequency of induced mutants increased to 1–2 mutants/10^6 CFC. P3 cells treated with MNNG (7 μM) seeded at 10^5 cells/60-mm petri dish yielded about 130 mutants/10^6 CFC. At lower cell densities, the frequency of induced mutants increased. With 5×10^4 cells/petri dish, 270 TG-resistant mutants/10^6 CFC were found, and at 2×10^4 cells/petri dish, 350 such mutants were produced. A further reduction in the cell density to a seeding level of 10^4 cells/petri dish, did not cause significant increase in the frequency of the TG-resistant mutants. In view of these results, in subsequent experiments, TG-resistant mutants were selected using a seeding level of 2×10^4 cells/60-mm petri dish.

In another series of experiments performed to determine the optimal time required by the newly mutated P3 cells to express their mutant phenotype, we analyzed the expression of TG resistance in P3 cells treated with either 3 or 7 μM MNNG, 0.1 μM BPDE, or 0.4 μM B[*a*]P activated in a cell-mediated assay by the human breast carcinoma BJ cells (Fig. 2). The results indicated that irrespective of the type of mutagen or its dose (in the case of MNNG) the number of TG-resistant mutants/10^6 CFC increased with time up to 6–7 days and remained constant for an additional 6–7 days (Fig. 2). At these optimal expression times, cells treated with either 3 or 7 μM MNNG yielded about 130 and 350 TG-resistant mutants/10^6 CFC, respectively. Cells treated with 0.4 μM B[*a*]P yielded about 50 mutants/10^6 CFC and those treated with 0.1 μM BPDE about 40 such mutants. At later times, as established for 7 μM MNNG-treated cells, these frequencies dropped with time (Fig. 2). On day 32 after treatment, the mutation frequency was reduced by more than 98% with only 5 TG-resistant mutants produced per 10^6 CFC. Thus, in order to obtain an efficient expression of TG resistance, mutants were selected in the subsequent experiments on the 7th day after treatment with the mutagen/carcinogen.

Characterization of induced TG-resistant mutants

To determine the stability of the mutant phenotype, we isolated 3 MNNG- and 6 B[*a*]P-induced TG-resistant mutants and tested their susceptibility to the cytotoxic effect of 4–120 μM TG after a subculture period of 4–6 weeks in the absence of the selecting agent (Table 1). The cloning efficiencies of the various mutant cells varied from 45 to 75%. No reduction in the cloning efficiencies of the mutant cells was observed when they were treated with TG at doses of 4–40 μM. Cells from three of the mutants (NG-3, BP-1, BP-5) exhibited some degree of susceptibility to the cytotoxic effect of 120 μM TG as detected by a reduction of about 30% in their cloning efficiencies. The cloning efficiency of the parental P3 cells was reduced by 97% when they were treated with 4 μM TG and by more than 99% when they were treated with 12 μM TG (Table 1).

To further characterize the phenotype of each mutant, we assayed HGPRT activity in cell lysates derived from 3 MNNG- and 3 B[*a*]P-induced mutants (Table 2). This enzyme activity was detected by measuring the generation of [^{3}H]IMP from [^{3}H]hypoxanthine. The results indicate that the parental P3 cell lysate yielded 4.6 pmoles

TABLE 1

SUSCEPTIBILITY OF PARENTAL AND MUTANT P3 CELLS TO THE CYTOTOXIC EFFECT OF TG

Cell type	Relative percentage of CFC after treatment with different TG concentrations (μM)			
	4	12	40	120
P3	2.8	0.3	< 0.03	< 0.03
NG-1	97	92	94	92
NG-2	92	93	100	95
NG-3	96	94	94	74
BP-1	101	100	97	67
BP-2	97	103	108	88
BP-3	98	102	95	87
BP-4	97	96	93	92
BP-5	98	95	99	73
BP-6	95	96	107	93

NG-1, -2, -3 cells are the progeny of TG-resistant colonies isolated from P3 cells after treatment with 7 μM MNNG and BP-1, -2 ... -6 from P3 cells treated with 4 μM B[*a*]P activated in a cell-mediated assay with BJ cells. The cells were used in the cytotoxicity studies after the mutant cells were subcultured for 4–6 weeks in the absence of TG. The cloning efficiency of the P3 cells was 69%, whereas the cloning efficiency of the mutant cells varied from 45 to 75%. These cloning efficiencies were considered as 100% for the relative percentage of CFC.

IMP/min/μg protein, whereas the HGPRT activity in the cell homogenates from each of the 6 mutant cell lysates was reduced by more than 90% from this level. 5 of the 6 mutants exhibited an HGPRT activity of 0.2–0.3 pmole IMP/min/μg protein.

In addition to measuring HGPRT activity, we analyzed the karyotype of the parental P3 cells and of cells from an MNNG- and a B[*a*]P-induced TG-resistant mutant. The results indicate that more than 80% of the parental and mutant cells had diploid karyotypes with a content of 46 (including XX) chromosomes (Table 3, Fig. 3). Less than 10% of cells in the parental and mutant cells exhibited a tetraploid or nearly tetraploid karyotype. The 3 cell types contained 2 marker chromosomes, which resulted from a translocation between chromosomes 15 and 20 (Fig. 3, Table 3). These marker chromosomes are also characteristic of the cell line from which the P3 clone was isolated (Zeuthen et al., 1980). The comparison between the karyotypes of the two TG-resistant mutant cells with the karyotype of the parental P3 cells indicated that the acquired resistance to TG was not associated with any detectable gross structural change in the X chromosomes in which the HGPRT gene is located (Caskey and Kruh, 1979) or in any of the other chromosomes.

TABLE 2

HGPRT ACTIVITY IN PARENTAL P3 AND 6 TG-RESISTANT MUTANT CELLS

Cell type	Specific activity (pmoles/min/μg protein)
P3	4.6 ± 1.0
NG-1	0.2 ± 0.1
NG-2	0.2 ± 0.1
NG-3	0.3 ± 0.1
BP-1	0.1 ± 0.1
BP-2	0.2 ± 0.1
BP-3	0.2 ± 0.1

The origin of the NG-1, -2, -3 and BP-1, -2, -3 mutants is described in Table 1. The results are the mean ± S.D. of 7 measurements.

Induction of TG resistance by carcinogenic PAHs activated in a human cell-mediated assay

In the absence of PAH-metabolizing cells, B[*a*]P, B[*e*]P, chrysene, DMBA, MCA, and pyrene

TABLE 3

MODAL CHROMOSOMAL NUMBER AND CHROMOSOMAL CHANGES IN PARENTAL P3 AND 2 TG-RESISTANT MUTANT CELLS

Cell type	Cells counted	Number of chromosomes									
		44	45	46	47	48	53	86 (tetraploidy)	87	90	92
P3	50	0	2	41	4	0	1				2 hr
NG-1	50	0	1	44	3	0			2		
BP-1	50	0	2	45	0	0		1		2	

	Number of metaphases analyzed	Karyotype
P3	8	46, XX, t(15p20p), t(15q20q), -15, -20
NG-1	8	46, XX, t(15p20p), t(15q20q), -15, -20
	2	46, XX, t(15p20p), t(15q20q), -15, -20, del(4)(q26)
BP-1	6	46, XX, t(15p20p), t(15q20q), -15, -20

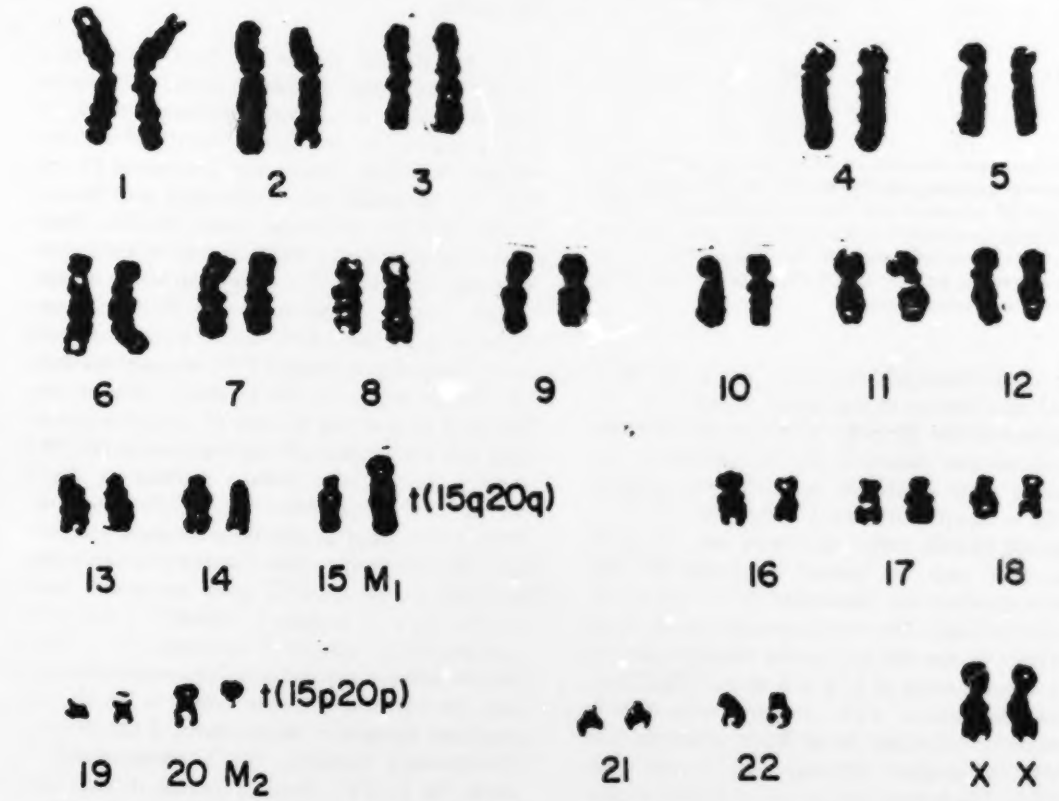

Fig. 3. Karyotype of untreated P3 cells.

TABLE 4

INDUCTION OF TG-RESISTANT P3 MUTANTS BY CARCINOGENIC PAHs ACTIVATED IN A HUMAN CELL-MEDIATED ASSAY

PAH	Concentration (μM)	Number of TG-resistant mutants/10^6 CFC
Control	-	2 ± 2
Pyrene	4	2 ± 2
	12	5 ± 2
B[*e*]P	4	3 ± 2
	12	7 ± 3
Chrysene	4	12 ± 4
	12	24 ± 5
B[*a*]P	0.15	26 ± 5
	0.4	47 ± 8
	1.3	103 ± 32
	4.0	124 ± 60
MCA	0.15	45 ± 12
	0.4	188 ± 25
	1.3	196 ± 30
DMBA	0.05	56 ± 21
	0.15	106 ± 18
	0.4	211 ± 26
	1.3	181 ± 47

2 days after treatment with DMBA, BP, and MCA, there were $0.3–1.6 \times 10^6$ cells/petri dish. The cloning efficiencies of these treated cells varied from 7 to 48%. In all other cases there were $1.4–1.8 \times 10^6$ cells/petri dish and the cloning efficiencies of these cells varied from 31 to 52%. The results are the mean ± S.D. of 4–6 measurements.

over a concentration range of 0.1–12 μM produced no cytotoxic or mutagenic response for TG resistance in the P3 cells. However, a concentration-dependent induction of TG resistance was detected after treatment with the carcinogenic PAHs — B[*a*]P, chrysene, DMBA, and MCA — when the P3 cells were cocultivated in a cell-mediated assay with the human carcinoma BJ cells (Table 4), which can metabolize B[*a*]P into water-soluble products. The most active carcinogen used, DMBA, was also the most active mutagen yielding at a concentration of 0.15 μM about 100 TG-resistant mutants/10^6 CFC compared with 45 such mutants for MCA and 26 for B[*a*]P, which are also potent carcinogens (although less potent than DMBA). The highest frequencies of mutant induction observed in the assay were approximately 200 TG mutants/10^6 CFC; these frequencies were detected after treatment with 0.4 and 1.3 μM DMBA and MCA. Chrysene, which is a weaker carcinogen than DMBA, MCA, or B[*a*]P, was also a weak mutagen yielding at a concentration of 12 μM a frequency of mutant induction equivalent to that of an 80-fold lower concentration of B[*a*]P. B[*e*]P and pyrene, which are less active as carcinogens, were also less effective as mutagens (Table 4).

In summary, P3 cells can be used in a human cell-mediated assay in which carcinogenic PAHs can be detected as mutagens, and the degree of induction of TG-resistant mutants in this assay is in accord with the observed carcinogenic potential of these chemicals in experimental animals (IARC, 1973).

Discussion

In the present studies we have developed a mutagenesis system utilizing a clonal cell population, derived from a human teratoma cell line, for use in testing the mutagenic activity of environmental chemicals. These cells, designated P3, exhibit an epithelial cell morphology and have a stable diploid karyotype with 46(XX) chromosomes, including a translocation between chromosomes 15 and 20. TG resistance, which is associated with a mutation at the HGPRT locus (Caskey and Kruh, 1979), can be induced in these cells. Analysis of a series of 6 TG-resistant mutants, induced by either MNNG or B[*a*]P, revealed that TG resistance in the mutant P3 cells was associated with a more than 10-fold reduction in HGPRT activity. Cell lysates from a number of the 6 mutants exhibited detectable HGPRT activity. These results suggest that these mutants probably did not result from a major deletion or a frame-shift mutation in the HGPRT gene but rather from another type of mutation, probably a base-pair substitution. In support of this suggestion, a comparison between two induced TG-resistant mutants and the parental P3 cells failed to reveal any consistent changes in the structures of the different chromosomes, including the X chromosomes in which the HGPRT locus is located (Caskey and

Kruh, 1979). In contrast to these results, induction of TG resistance by X-rays in human fibroblasts involved deletions in the X chromosomes (Cox and Masson, 1978).

In addition to characterizing the properties of TG-resistant mutants, we determined the conditions under which such mutants can be efficiently selected. These included seeding the P3 cells prior to selection at a cell density of 2×10^4 cells/60-mm petri dish to reduce the effect of metabolic cooperation among cells and allowing the newly mutated cells an expression time of 7 days in order to eliminate from the mutants the functional wild-type enzyme molecules via cell division, dilution, and/or degradation (Bradley et al., 1981; O'Neill et al., 1977; Van Zeeland et al., 1972; Van Zeeland and Simons, 1976). Other human cell types can have phenotypic expression times for the optimal expression of the resistance that differ from that determined for P3 cells (Jacobs and DeMars, 1978; McCormick and Maher, 1981; Michalopoulos et al., 1981; Thilly et al., 1980). An interesting observation was the reduced detection of TG resistance at expression times longer than 2 weeks. Similar observations at prolonged expression times were described also by other authors in human and Chinese hamster cells (Bonatti et al., 1978; Diamond et al., 1982; Jacobs and DeMars, 1978). It was suggested that the decreased detection of the TG-resistant mutants is the result of their growth disadvantage relative to the parental cells (Bonatti et al., 1978; Diamond et al., 1982).

Using the conditions established for the efficient detection of TG-resistant mutants, we tested the mutagenic response of a series of 6 PAHs in the P3 cells. Like other types of cells used in mutagenesis studies, the P3 cells are not capable of activating many proximate mutagens/carcinogens (Bigger et al., 1980; Huberman and Sachs, 1974). To detect the mutagenic activity of these chemicals, P3 cells have to be supplemented with an exogenous metabolic activation system. We have therefore cocultivated the P3 cells in a human cell-mediated assay (Aust et al., 1980; Diamond et al., 1980; Gould et al., 1982, Harris et al., 1978; Huberman and Sachs, 1974) with BJ carcinoma cells. Preliminary data indicated that the BJ cells are capable of metabolizing B[*a*]P into water-soluble products (unpublished results). In this human cell-mediated assay, TG-resistant mutants were induced by DMBA, MCA, B[*a*]P, and chrysene; the degree of mutant induction was related to the carcinogenic potency of the PAH (IARC, 1973). Pyrene and B[*e*]P, which are not considered to be carcinogenic, were either inactive or exhibited a limited induction of TG resistance. Preliminary studies have also shown that P3 cells can be used to detect the mutagenic response of B[*a*]P in a cell-mediated assay with Syrian hamster embryo cells (Huberman and Sachs, 1974) and of dimethylnitrosamine in an assay with rat-liver hepatocytes (Jones and Huberman, 1980; Langenbach et al., 1978a).

In summary, our results indicate that the P3 cells derived from a human epithelial teratocarcinoma cell clone can be useful in detecting mutations induced at the HGPRT locus by direct-acting and proximate mutagens/carcinogens under appropriate assay conditions.

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